THE OPTIC NERVE FIBRES AND GANGLION CELLS OF THE MAMMALIAN RETINA. By George Thin, M.D. (Plate XIII.)

The isolation of the ganglion cells and optic nerve fibres of the retina, has certainly not been found by histologists to be invariably an easy task, and I can testify from experience that methods which are well fitted for the observation and study of other parts of the retina destroy the processes of the ganglion cells and the nerve fibres. Max Schultze has acknowledged this difficulty. In his article on the retina in Stricker's *Handbuch*, published in 1872, he makes the following remarks:—"Notwithstanding *the difficulty of isolating the cells in a good state of preservation*," he observes, "there are nevertheless a complete series of observations regarding long and branching processes which are given off in the same way as the processes of the ganglion cells of the central organs. The exact conditions of maceration of the retina necessary to secure this successful isolation, appear, however, not to be easy of attainment."

I am induced, therefore, to believe that the publication of a method by which I have found the isolation of these elements singularly easy, may be considered justifiable. At the same time, the description of the appearances observed, whilst engaged in studying the isolated cells, will, I hope, be of some use as a contribution to the evidence already existing regarding certain points on which histologists still hold considerably divergent opinions.

The method I have followed holds good for the retina of the cat and the sheep; but there can be little doubt that it will prove equally useful in the case of many other mammalia. My observations have been limited to the eyes of these two animals.

It is well known that if a sheep's eye be placed entire in a sufficient quantity of alcohol for twenty-four hours, and at the end of that time be laid open and the retina be then examined in glycerine, the optic nerve fibres and ganglion cells will be found more or less well preserved. But it is a matter of no small importance to regulate the strength of the alcohol, and diluted alcohol will be found more useful than strong alcohol. A mix-

ture of equal parts of methylated alcohol and water is a strength that I used for some time with such excellent results that I adhered to it during most of the time that I was engaged in examining this part of the retina; but latterly I found that, in most respects, a weaker strength secured as good preparations, and, for some purposes, produced better ones. For the preservation of the processes of the ganglion cells, mixtures of one part of methylated alcohol with two of water, and of one of methylated alcohol with three of water, are peculiarly well adapted. The fibres of the optic nerve expansion are well seen whichever of these strengths is used. They may be isolated in great numbers and for great lengths, after the bulb has been in equal parts of water and alcohol. When only a fourth strength of alcohol was employed, the nerve fibres were, unless well teased out, slightly obscured by adherent granules—probably the remains of connective substance of the layer.

When the strengths of a third and a fourth were used, the bulb was allowed to remain in the fluid for 36 or 48 hours.

Although both the ganglion cells and the nerve fibres in eyes, treated by the above methods, can be examined at once in glycerine, it may be found advantageous to subject the retina to other processes through which the hardened nerve-elements can now pass without injury. It may be placed first in water for a short time, and then may remain over night in staining fluids, and finally be examined and preserved in glycerine, or, after being stained, it may be passed through alcohol and oil of cloves and preserved in dammar-varnish. The glycerine preparations show both the fibres of the optic nerve expansion and the ganglion cells. The dammar preparations are useful as permanent specimens of the nerve fibres. In either case some careful manipulation with needles is necessary to disentangle the nerve fibres—a process which is particularly troublesome in the dammar preparations. Of all the staining fluids which I tried, I found a solution in water of aniline blue by far the best. For the nerve fibres aniline blue alone is sufficient; for the ganglion cells a double staining with aniline blue and eosin is useful.

Eyes which have been placed in alcohol, as above directed, may be preserved for a long period in glycerine without the

nerve fibres or ganglion cells suffering in the least. The effect of the glycerine by its affinity for water is to produce a complete collapse of the eyeball. The lens preserving the shape of the anterior part of the bulb, the posterior half is doubled up into the anterior half, forming a cavity at the bottom of which is the stump of the optic nerve. It is thus possible to prepare eyes at any time, and keep them ready for examination. I had excellent preparations of the optic nerve fibres and ganglion cells from the eye of a kitten, which, after being 24 hours in equal parts of methylated alcohol and water, had been kept 16 months in glycerine.[1]

In the sheep a very large proportion of the nerves are exceedingly fine, being similar to the smallest nerves in the illustration by Max Schultze at page 982 of Stricker's *Handbuch*. The varicosities, as I observed them, are for the most part exactly similar to those described, and figured by that histologist. But this was not invariably the case. The usual appearance is that shown in fig. 1, which represents a large fibre with varicosities, the whole fibre being stained uniformly by aniline blue. In fig. 2, a different appearance has been drawn. In this fibre and other similar ones which I observed, the varicosities have a special structure which I have not seen noticed before. The deeply stained fibre is continued through the centre of the varicosity, the peripheral parts of which are feebly stained, and have a thin membranous appearance.

An analogous appearance is seen in the fine fibres. In these the varicosities are of two kinds. There are numerous minute oval thickenings in the fibre which stain deeply with aniline blue, and the finer the fibre the more numerous are these thickenings. The part of the fibre between two swellings remains perfectly colourless. In some of the finest fibres the colourless thread is scarcely visible with a power of 400 diameters, the

[1] The method is one that might be used for the examination of the retina of rare animals when the eyes have to be procured from a distance. After the remarkable observation of the anastomosis of the ganglion cells of the elephant's retina by Corti, to which there has been, as yet, no parallel, it seems to me that a further examination of the retina of that animal is very desirable. The eyes of elephants in a condition suitable for such an examination are not easily procurable, but by the use of the above method available specimens might be had from India.

course of the fibre being marked by a line of deeply stained varicosities. The difference is more than one of degree, or than can be accounted for by the greater volume of the fibre at these points, and can, it seems to me, only be explained by supposing that there is accumulated in these swellings a substance which no longer at least exists in the intermediate parts of the fibre.

The varicosities on these fine fibres are seen in another form.

The fibre appears to open out for a short extent, so as to enclose a more or less elliptical or oval space. In this space a very finely granular substance can be observed. Sometimes the fibre remains perfectly straight and this space bulges out on one side like a pouch. A common form of this pouch is shown at the left extremity of fig. 4. The contents of the space do not stain by aniline blue.

I have called special attention to these varicosities which do not stain in order to describe a peculiarity which I have observed in some of them, and which I have shown in figures 3 and 5. In a few instances I have been able, as is seen in the drawing, to trace the minute fibre through the centre of the varicosity, the appearance so produced having a certain analogy to that shown in the larger fibre drawn in fig. 2.

These appearances bear directly on the question, whether the nerve fibres in the optic nerve expansion are, as they are usually described, naked axis-cylinders or whether they are not invested by a delicate membrane, but I concede that they are far from sufficient to settle it. Although the formation of varicosities takes place after death, and is in the present case determined as to its special form by the re-agents used, yet the constancy with which the same changes are observed suggests that they are determined by some structural peculiarity.

As compared with the retina of the sheep, a large proportion of broad fibres are found in the retina of the cat, and as the cat's retina has not been so much an object of study as that of the sheep or ox, I have drawn examples of the fibres which are mostly found in it, in figures 6, 7, and 8.

The ganglion cells shown in figures 9–23 have been drawn partly to show their various forms and sizes, and partly because they illustrate interesting points in connection with the cell processes.

In regard to the question as to whether all the ganglion cells have processes, I believe that preparations obtained by the methods which I have described, will settle it definitely in the affirmative. I have not observed a single cell to which there were not processes or distinct remnants of processes attached. In many of the smaller cells, however, the processes are so fine that I can easily imagine how they might completely disappear under the action of re-agents or in manipulation. In figures 14, 15, 22, and 23, I have drawn examples of such cells. An example of the now well-established dichotomous division of the process that joins the fibres of the optic nerve expansion is shown in figure 11.

I have observed appearances in a large number of cells that are only explicable on the theory that all the processes are enveloped in a connective tissue sheath, which is continuous with the surface of the cell—even the optic nerve process, which is usually seen and has been always described as straight and smooth. It is to this sheath that I attribute the granular and slightly fibrillated appearance of the large broad processes that pass outwards towards the molecular layer. Examples of this appearance in cells from the cat's retina are shown in figures 12 and 21. In figure 17 I have drawn a cell from the cat's retina, in which an empty sheath was attached to the surface of the cell. I describe this appearance as that of an empty sheath, because it differed from the usual processes in being collapsed and slightly torn. This collapsed appearance and its origin from and connection with the surface of the cell were very striking in the preparation, although they have been insufficiently reproduced in the drawing. The appearance was several times observed.

In many cells the processes were distinctly granular up to a certain point, whence an even varicose nerve fibrilla emerged, contrasting with the granular substance which lay between it and the cell. Examples of processes in this form are shown in figure 18, a drawing of a ganglion cell from the retina of the sheep. The true nerve elements in the processes of this cell are, as I believe, the varicose fibres in which the processes are continued. In the parts of the processes between the cell and the fibrillæ it is the connective tissue surrounding the fibrillæ

which is seen. In figure 14 a cell is drawn in which three processes in the form of simple nerve-fibrillæ leave the cell, a fourth process still retaining the fine granular sheath. Figure 22 illustrates the same point.

In figures 10, 12, 21 (*a*), and probably also 17 (*a*), examples are shown of the optic nerve process being covered with a sheath for some distance after it has left the cell. In figure 12 (*a*) the sheath had become detached from the fibre except at the point of origin of the latter. The torn empty sheath twisted round the root of the fibre and floated lightly in the preparation alongside of it.

In figure 19 a cell is drawn in which a fibre—to all appearance the optic nerve fibre—passes straight into the substance of the cell, the sheath in which it lies being continuous with the surface or wall of the cell. In figure 13 a cell is shown, in which the optic nerve fibre penetrated the cell and reached the nucleus, but whether it actually touched the nucleus I could not certainly make out.

Figure 9 is drawn because it is the only purely bipolar cell similar to those figured by Max Schultze (loc. cit. p. 986) which I saw in the cat's retina, amongst a considerable number of cells of very various forms which I observed. Figure 16 is drawn on account of the length of the processes isolated; and figure 20 simply in illustration of one of the characteristic forms of the cells in the cat's retina.

Explanation of Plate XIII.

Optic Nerve Fibres.

Figs. 1, 2, 3, 4, and 5, are drawings of optic nerve fibres from the retina of the sheep; figs. 6, 7, and 8 from the retina of the cat. They were all (with the exception of figs. 4 and 5) drawn by camera lucida, and are magnified 700 diameters. Fig. 4 is magnified about 300, and fig. 5 about 400 diameters; but the magnifying power in these two figures must be taken as having reference only to the larger varicosities, the fibres themselves being too fine to be accurately drawn as regards breadth.

Ganglion Cells.

Fig. 9. From the cat's retina. Bulb 48 hours in equal parts of methylated alcohol and water, and then three months in glycerine. × 260 (camera).

Fig. 10. From the cat (same retina as fig. 9). × 260 (camera).

Fig. 11. From the sheep's retina. Bulb in a mixture of one part methylated alcohol and two of water for 36 hours. × 500 (camera).

Fig. 12. From the cat (same retina as fig. 9). *a*, Sheath of optic nerve fibre. × 260 (camera).

Fig. 13. From the cat (same retina as fig. 9). Hartnack obj. 8, eyep. 3. Tube in.

Fig. 14. From sheep's retina. Bulb 36 hours in a mixture of one part methylated alcohol and two of water. Hartnack obj. 8, eyep. 3. Tube in.

Fig. 15. From the cat (same retina as fig. 9). × 260 (camera).

Fig. 16. From the cat (same retina as fig. 9). Hartnack obj. 8, eyep. 3. Tube in.

Fig. 17. From the cat (same retina as fig. 9). Hartnack obj. 8, eyep. 3. Tube in.

Fig. 18. From the sheep's retina. Bulb 24 hours in a mixture of one part methylated alcohol in two of water. The retina then removed and placed 12 hours in water. Examined in glycerine. Hartnack obj. 8, eyep. 3. Tube in.

Fig. 19. From the cat (same retina as fig. 9). Hartnack obj. 8, eyep. 3. Tube in.

Fig. 20. From the cat (same retina as fig. 9). Hartnack obj. 8, eyep. 3. Tube in.

Fig. 21. From the cat (same retina as fig. 9). *a*, Optic nerve sheath. × 260 (camera).

Figs. 22 and 23. From sheep's retina. Bulb 36 hours in a mixture of one part of methylated alcohol in two of water. Hartnack obj. 8, eyep. 3. Tube in.

11 A.

ON THE MOVEMENTS OF THE IRIS. By William Ackroyd, F.I.C., &c.

Sect. I. It is well known that the movements of the iris are due to the stimulus of light, but I am not aware that any experiments have been hitherto made to determine the approximate quantity of that agent necessary to bring about this involuntary action. The usual way of observation precludes refined experimenting, it being customary to watch the iris of another person or animal whilst under the influence of varying amounts of light, or one's own iris by means of a mirror. Three methods will be described here, and I believe that one at least may afford a means of getting new data on this and other points.

Sect. II. The first and second methods depend upon the following facts:—that, if a divergent bundle of rays emanate from a small surface or hole, very near to the eye (say about 30 mm. off), this surface or hole is the apex of a cone of light whose base is the pupil; that every movement of the iris affects the area of this base, which appears as a circular luminous field; and finally, that I find these alterations of area, so easily seen, may be taken as indications of the movements of the iris.

The third method is equally simple. The lachrymal fluid on the surface of the cornea affects the image of any light source such as a lamp or star, and by refraction causes the appearance of rays to emanate therefrom.

It is obvious that the length of these rays must be regulated by the iris, this organ being nearer to the retina, hence when the pupil contracts the rays ought to shorten, and when the pupil expands the rays ought to lengthen out. Such I find to be the case.

Sect. III. *The First or Reflection Method.*—The following is the simplest form of the experiment I have been able to devise. Burnish the head of an ordinary brass pin, and place the pin up to head in a black hat. Now with one eye shut and your back to the light, bring this pin head near to the other eye so that

the light may be reflected into it from the convex surface of the pin-head.

One sees a circular luminous field, with projecting hairs at the bottom which belong to the top eyelid.[1] Globules of the lachrymal fluid also appear at each wink.

Expt. 1. Shade the light from the observing eye for a few seconds, then let the light fall on it again. Notice the alteration in area of the field of view. The field contracts, then expands slightly, and oscillates until the iris is adjusted for the amount of light falling into the eye.

Expt. 2. Observe the pin-head with the right eye for some moments, the left eye being closed. Open the left eye. The iris of the right eye is seen to move markedly, the pupil contracting. *Here the iris of the right eye is moved by the light entering the left one.*

Expt. 3. With everything as in Expt. 2, have both eyes closed and only open the right or observing eye. There is contraction of the pupil, but apparently no more marked than in Expt. 2.

Sect. IV. *The Second or Transmission Method.*—Prick a pin-hole in tin foil. Shut one eye and bring the hole within 12 mm. off the open eye.

Expts. 1 2, and 3 may readily be repeated by this method.

Expt. 4. Place green glass before the aperture and notice the size of the field, then withdraw the glass suddenly. The pupil contracts. Red glass gave the same result.

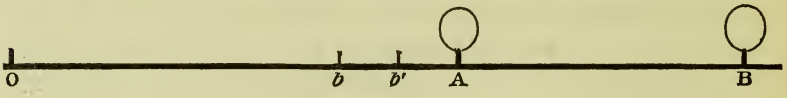
Sect. V. *The Third or Refraction Method.*—The following example will make perfectly clear the way of working here :—I am looking at a star, with the moon at full, a little to one side. From the star proceed the rays mentioned at the close of Sect. 2. Upon turning towards the moon, but still keeping my attention concentrated on the star, the rays of the latter appear to retreat into it ; and upon turning from the light of the moon, the rays emanate from the star again.

Expt. 5. This is typical of about seventy other experiments I have made. The night is starless. An isolated gas lamp, with

[1] A simple method is here suggested for demonstrating to one's self the inverting action of the crystalline lens. With everything as here described, take a needle and bring it across the field of view *close* to the eyelids. *If it move downwards it appears to move upwards ; if it be moved upwards it appears to come downwards.*

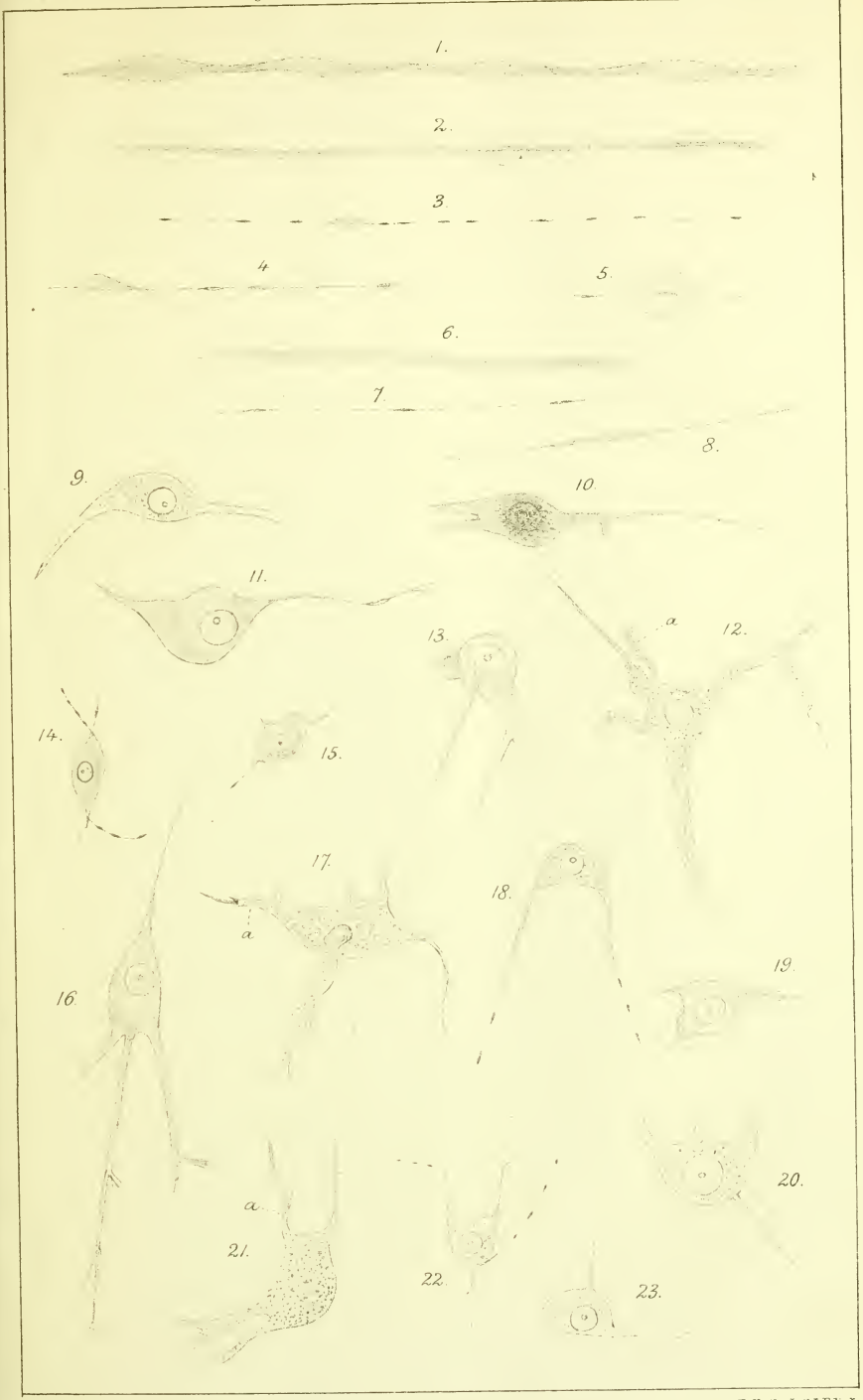
no houses near or any other sources of light, appears, when seen from a distance, with the usual rays emanating from it. I walk towards it slowly. At 300 yards no alteration has taken place in the rays; they appear fixed. The distance is slowly decreased, but not until I am at a distance of 16 yards do the rays perceptibly shorten—in other words, the light from this one gas lamp is incompetent to effect a movement of my iris until I am within 16 yards of it. The shortening of the rays is now rapid, for at 10 yards distance the light appears to be without them.

Expt. 6. In the preceding experiment there is a possibility that the rays may be shortened to some extent by the increase in size of the image on the surface of the cornea as we near the light. In the present experiment this objection is to some extent removed. Two gas lamps were chosen, 50 yards apart, and whilst walking towards the nearest my attention was kept exclusively on the rays emanating from the furthest one. As the first lamp is approached, the effect of its light on the iris is visible in the alteration of length of rays proceeding from the far one. Thus in the accompanying fig. A, the two lamps are A and B, and the observer stationed at O sees rays emanating from both. A is the lamp whose influence on the iris is to be tested, and B is the lamp light used as a tester. Proceeding from O towards B, a point *b* is reached at which the lamp rays of B begin to shorten, *i.e.*, the light of A affects the iris. Getting nearer still to A a point *b'* is reached, where the distant light B appears to have lost its rays.



The average of a dozen experiments gave as the value of *b*A... 14 yards, and as the value of *b'*A... 8 yards. Squaring these numbers it appears that about one-third of the light competent to contract the pupil very markedly is sufficient to start its movement.

At present, I abstain from comment, as I am continuing these experiments.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